

Serial No.: 09/954,737
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Amendments to the Specification

Please replace the Sequence Listing on pages 1-9 as filed on June 4, 2004, with a substitute Sequence Listing presented on pages 1-24, enclosed herewith.

On page 5, please replace the paragraph starting on line 4 with the following:

Fig. 1A-B. **Tat components of *B. subtilis* and *E. coli*.** The amino acid sequences of Tat components of *B. subtilis* and *E. coli* as deduced from the SubtiList (<http://bioweb.pasteur.fr/Genolist/SubtiList.html>) and Colibri (<http://bioweb.pasteur.fr/Genolist/Colibri.html>) databases were used for comparisons. Identical amino acids, or conservative replacements are marked. Putative transmembrane segments, indicated in gray shading, were predicted with the TopPred2 algorithm (34, 35) (A) Comparison of TatAc (YnzA, SEQ ID NO:5), TatAd (YczB, SEQ ID NO:4) and TatAy (YdiI, SEQ ID NO:3) of *B. subtilis* (Bsu) with TatA (SEQ ID NO:1), TatB (SEQ ID NO:6) and TatE of *E. coli* (Eco) (SEQ ID NO: 24-6). (B) Comparison of TatCd (YcbT, SEQ ID NO:9) and TatCy (YdiJ, SEQ ID NO:8) of *B. subtilis* with TatC of *E. coli* (SEQ ID NO: 7[[[-9]]]).

On page 7, please replace the paragraph starting on line 14 with the following:

Fig 7. **Predicted twin-arginine (RR-)signal peptides of *B. subtilis*.** The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X-ΦΦ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (ie. positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

Please replace Table I and the text following the Table, on page 56 with the following:

Table I. Predicted Twin-Arginine Signal Peptides of *B. subtilis**

| protein | signal peptide | SEQ ID NO |
|--------------------------------|--|-----------|
| AlbB | MSPAQRRLLYILSFIFVIGAVVYFVKSDYLFTLIFIAIALF | 84 |
| AmyX ^T _M | MVSIRRSFEAYVDDMNIITVLIPAEQKEIM | 53 |
| AppB TM | MAAYIIRRTLMSIPILLGITLSFVIMKAAPG | 54 |
| LipA | MKFVKRRRIALVTILMLSVTSLFALQPSAKAAEH | 55 |
| OppB TM | MLKYIGRRLVYMIITLFVIVTVTFFLMQAAPG | 56 |
| PbpX | MTSPTRRRRTAKRRRRKLNKRGKLLFGLLAVMVCITIWNALHR | 57 |
| PhoD | MAYDSRFDEWVQKLKEESFQNNTFDRRKFIQGAGKIAGLSLGLTIAQSVG AFEV | 58 |
| QcrA | MGGKHDISRRQFLNYTLTGVGGFMAASMLMPMVRFA | 59 |
| SpolIJ | MLLKRRIGLLLSMVGVMFLLAGCSSV | 60 |
| TipA TM | MKKTLTITIRRSSIARRLIISFLLILVPIITALSVSAYQS | 61 |
| WapA | MKKRKRRNFKRFAAFLVLALMISLVPADVLAKST | 62 |
| WprA | MKRRKFSSVVA AVLIFALIFSLFSPGTKAAAAGA | 85 |
| YeeA TM | MEMFDLEFMRRRAFLAGGMIAVMAPILGVYLVLRQ | 64 |
| YdeJ | MKKRRKICYCNTALLLMILLAGCTDS | 65 |
| YdhF | MRRILSILVFAIMLAGCSSN | 66 |
| YdhK | MSAGKSYRKKMKQRRMNMKISKYALGILMLSLVFLSACGNNN | 67 |
| YesM TM | MKKRVAGWYRRMKIKDKLFVFLSLIMAVSFLFVYSGVQYAFHV | 86 |
| YesW | MRRSCLMIRRRKRMFTA VTLVLLVMGTSVCPVKAEGA | 69 |
| YfkN TM | MRIQKRRTHTVENILRILLPPIMILSLILPTPIHAEEES | 70 |
| YkpC | MLRDLGRRVVAIAAILSGIILGGMSISLANMP | 71 |
| YkuE | MKKMSRRQFLKGMFGALAAGALTAGGGYGYARYL | 72 |
| YmaC | MRRFLLNVILVLAIVLFLRYVHYSLEPE | 73 |
| YmzC | MFESEAE LRRIRIALVWIAVFLLFGACGN | 74 |
| YubF TM | MQKYRRRNTVAFTVLAYFTFFAGVFLFSIGLYNADNL | 75 |
| YuiC TM | MMLNMIRLLMTCLFLLAFGTTFLSVSGIEAKDL | 76 |
| YvhJ | MAERVVRVRVKKKKSKRRKILKRIMLLFALALLVVVGLGGYKLY | 77 |
| YwbN | MSDEQKKPEQIHRRDILKWGAMAGAAVAIGASGLGGLAPLVQTAAKP | 78 |

*Putative twin-arginine signal peptides were identified in two ways. First, the presence of the consensus sequence R-R-X-Φ-Φ (Φ is a hydrophobic residue), immediately in front of an amino-terminal hydrophobic region as predicted with the TopPred2 algorithm (34, 35), was determined. To this purpose, the first 60 residues of all annotated proteins of *B. subtilis* in the SubtiList database (<http://bioweb.pasteur.fr/Genolist/Subtilist.html>) were used. Second, within the group of twin-arginine membrane sorting signals, cleavable signal peptides were identified

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with the SignalP algorithm (61, 62). Conserved residues of the twin-arginine consensus sequence R-R-X-Φ-Φ) are indicated in bold. In addition, positively charged residues that could function as so-called Sec-avoidance signal (54) are indicated in bold and italics. The hydrophobic H-domain is indicated in gray shading. In signal peptides with a predicted signal peptidase I cleavage site, residues from position -3 to -1 relative to the signal peptidase I cleavage site are underlined. Notably, some of these proteins contain one or more putative transmembrane segments elsewhere in the protein (indicated with "TM"), or are putative lipoproteins. Residues forming a so-called lipobox for signal peptidase II cleavage are enlarged in size.

Please replace Table IV and following text, on page 59, with the following:

Table IV. Twin-Arginine Signal Peptides of PhoD and PhoD-like proteins*

| protein | signal peptide |
|---------------|--|
| PhoD (Bsu) | MAYDSRFDEWVQKLKEESFQNNRFD RRKFI QGAGK IAGLSLGLT IAQSVGA FEV (<u>SEQ ID NO:52</u>) |
| SP1 (Sco) | MTPAN HQAPT SAPSPAPSQSSHAPELRAAARSLG RRRFL TVTGAAAALAFAVN LPAAGTA |
| SP2 (Sco) | MAP TGRPS ALAEHAFSPHDAVLGAAARHLG RRRFL TVTAAAAALAFSTNLPA |
| SP3 (Sco) | RGAVA AP E (<u>SEQ ID NO:54</u>) |
| SP4 (Ste) | MTSRHRASENSRTPSRRT VV KAAAAGAVLAAPLAAALPAGA ADA APA (<u>SEQ ID NO:55</u>) |
| | MTPAARPSQHAP EL RAAARHLG RRRFL TVTGAAAALAFAVN LPAAGT AAAAEL (<u>SEQ ID NO:56</u>) |

*Homologues of *B. subtilis* PhoD were identified by amino acid sequence similarity searches in GenBank using the Blast algorithm. SP1 (Sco), gene Scc75A.32c of *Streptomyces coelicolor* (CAB61732); SP2 (Sco), gene SCF43A.18 of *S. coelicolor* (CAB48905); SP3 (Sco), gene SC4G6.37 of *S. coelicolor* (CAB51460), and SP4, phoD gene of *Streptomyces tendae* (CAB62565). GenBank Accession numbers are indicated in parenthesis. Conserved residues of the twin-arginine consensus are indicated in bold. The hydrophobic H-region is indicated by boxed text. Signal peptidase I recognition sequences predicted with the SignalP algorithm (61, 62) are underlined.